NOTES ON SOME PARASITIC PROTOZOA.

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(From the Bureau of Microbiology, Sydney, New South Wales.)

(Plate xlviii.)

The recently created Bureau of Microbiology is now undertaking a systematic search of the Australian fauna, both domesticated and native, with a view to finding out what parasites occur here. So far, probably two hundred specimens have been examined for hæmatozoa, and about the same number for intestinal parasites. Some of the results have already been made known in other publications. It is hardly necessary to point out that in the majority of cases parasites, especially those which infest the blood, were absent. Some of those which we have seen are now discussed in this paper.

Leucocytozoon (Hæmogregarina) muris Balfour.

(Plate xlviii., figs. 1-11.)

In 1906, one of us(J.B.C.)(1) while examining the blood of various rats in Perth, West Australia, noticed that the mononuclear leucocytes of *Mus alexandrinus* Geoff., were parasitised by an organism similar to that described by Balfour(2) from *Mus decumanus* Pallas, from the Sudan as *Leucocytozoon muris*. These organisms from Perth were recorded by mistake as *L. balfouri* and *L. ratti* from *Mus decumanus*. The rats were subsequently identified by Oldfield Thomas, on specimens being forwarded to the British Museum, as being *Mus alexandrinus* Geoff.

Quite recently the other of us(T.H.J.)(3) found the same parasite in two rats (*Mus decumanus*) caught in Sydney, and brought into the Bureau (along with many others) for examination. The

organism in question is probably rare, since, although over a hundred blood-films from rats and mice have been examined here, on no other occasion has it been detected, though *Trypanosoma lewisi* Kent, is not uncommonly met with here in *M. decumanus*, *M. rattus* and *M. alexandrinus*.

As was mentioned by Balfour, the parasite infests the extra-nuclear part of the mononuclear leucocytes. He found it in the blood from the heart, liver, and spleen, but not from the kidney and bone-marrow. This agrees exactly with what we have seen, though smears from the spleen showed very few forms. Encapsuled hæmogregarines (figs. 10-11) were present in the plasma from the heart and liver, having been set free by the mechanical destruction of the parasitised cell in making the smears. Unencapsuled free forms were not seen, nor were any segmenting stages (schizogony) detected in sections of the liver, kidney and spleen in both West Australian and Sydney specimens. Only a few young parasites were seen (fig. 8). They did not possess a capsule. The shape was mostly slightly crescentic; both extremities were blunt, one usually being somewhat wider. The nucleus was situated either centrally or a little nearer one end.

The commonest type was a more or less oblong encapsuled form representing an adult sporont. The size was from 6-10μ long by 2-5 wide. There is thus a greater variation in size than that noted by Balfour, who stated the measurements to be between 9 and 10·5μ long by 4·5 broad. As a rule the parasites were not crescentic, though such forms were not rare (fig. 10). The usual shape was that of a fairly solid body, the middle of which was as wide or only slightly wider than each end, the ends being of the same size and very bluntly rounded off (figs. 4-7). In one instance the organism closely resembled in form certain hæmogregarines from the erythrocytes in snakes, there being a somewhat wider anterior end gradually tapering into a narrow posterior end which was bent round to form a "tail" (fig. 3), the whole animal being surrounded by a wide capsule. Another fact worth noting in reference to this specimen, which was seen in a smear from the liver, is that the host-nucleus was divided into two quite separated portions.

The nucleus of the adult parasite was seen as a large conspicuous mass (Giemsa's stain), generally centrally situated, though in a few instances it was placed very obliquely. The capsule was usually quite distinct, there being in most specimens a considerable space between it and the enclosed parasite.

Commonly the organism occupied nearly all the extranuclear part of the leucocyte. Its position in the host varied considerably, though the most usual position was parallel to the inner side of the somewhat bean-shaped host-nucleus (fig. 6). At other times the parasite was obliquely, and in a few cases transversely, placed (fig.2). The host-nucleus was occasionally indented, especially by the transversely situated forms (fig.4), and in a few cases was actually divided (fig.3).

Schaudinn has stated that a leucocytozoon is a stage in the life cycle of a spirochaete or a trypanosome. If this be correct, which seems to us to be improbable, may *L. muris* and *Trypanosoma lewisi* be different phases of one organism?

In the films from one of the infected rats, both *L. muris* and *T. lewisi* were present, whilst in the other there were no trypanosomes.

HALTERIDIUM NETTIONIS, sp.nov.—A blood-parasite of *Nettion* (*Anas*) *castaneum* Eyton.

(Plate xlviii., figs.15-17.)

An examination of blood-films from an Australian teal, *Nettion castaneum* Eyton, New South Wales, prepared by the Director of the Bureau in 1907, and handed to us for examination, revealed the presence of Halteridia. These differ from the other Halteridia (*H. chrysops*, *H. meliornis*, *H. geocichlæ*, *H. philemonis*), which we have described(4) from Australian birds, in the large size of the parasite as regards the host-cell, and by the number and large size of the melanin-granules. The parasites were not numerous, but two types could be distinguished, extremes of which almost certainly represent the sexes, the intermediate types perhaps the schizonts.

(*a*) *Male gametocytes* (fig. 15).—Protoplasm shows very pale staining or none at all. In one there were thirteen masses of melanin, larger than in the female type, and, though fairly evenly distributed, grouped more towards the poles of the host-nucleus. The host-nucleus was distinctly displaced. In another, the melanin granules were similarly large, fairly evenly distributed, though tending to form three groups, one towards each end and one in the middle, the nucleus not as yet displaced.

(*b*) *Female gametocytes* (fig. 16).—Protoplasm decidedly stained. In two specimens, the pigment appeared as twenty-four to thirty masses, smaller than in the male form but larger than we have seen in other Australian Halteridia, more or less uniformly distributed, though in one with a tendency to be more numerous towards the end of the parasite. The host-nuclei in these were pushed to an almost lateral position, and the parasites occupied the whole of the host's protoplasm save a narrow band on the distal side of its nucleus. In another example, the pigment was more aggregated into three groups towards the two ends and in the middle of the parasite, the parasite was not quite so large, and the host-nucleus less displaced.

(*c*) The only intermediate form seen (fig. 17), perhaps a three-quarter grown schizont, had the protoplasm staining intermediately between the other two forms, with smaller granules at each end and a few in the middle.

The size of the corpuscles of this bird were 12·5 by 7μ, the nuclei of the corpuscles 5 by 3μ, and the parasite 18 (measuring along the middle and around the bends) by 4μ.

Blood-films from two birds were examined, the parasites occurring only in one.

We propose for this parasite the name *Halteridium nettionis*, the specific name being derived from the generic name of the host.

The type-slide has been presented to the Australian Museum, and the co-types are being retained by the Bureau.

PLASMODIUM PASSERIS, n.sp., from the Sparrow, *Passer domesticus*.

(Plate xlviii., figs.19-24.)

In March, 1909, a sparrow fell dead from its roosting-place one evening, and was picked up and submitted for examination by Mr. R. Grant, of the staff of the Bureau. Blood-films showed the presence of Plasmodia. In the early stage, these appeared as small amœboid masses, of slightly irregular outline, faintly stained bluish with Giemsa, and situated at or quite near one end of the host-cell. In double infections of one cell, the two parasites were found together at one end, one lying usually a little more laterally to the other, or at opposite ends of the host-cell.

As they increased in size, small melanin-granules became discernible, and the nucleus of the red corpuscle was gradually displaced to one side to make room for large, more or less spherical, bodies. Eventually, in the older forms, the host-nucleus had been extruded, the rounded parasite occupying its place in the distorted red cell. Finally, some examples of this stage of the parasite were found free in the plasma. In these large forms, one or two vacuoles were occasionally to be seen, and melanin in varying amounts was present in all. This pigment seemed to occupy no particular position, being scattered irregularly as small granules or aggregated in larger masses in various situations.

Two types could be distinguished in the large parasites: a very pale form and a well-stained, somewhat granular form. Apparently these represent, the former male gametocytes, the latter female. No definite difference in the amount or disposition of the pigment could be detected between the two forms. No parasites were seen in any stages suggestive of schizony.

The following is a description of ten consecutive full-sized parasites, arranged according to the types presented:—

(1) Very pale. Host's nucleus has disappeared. A few small scattered grains of melanin.

(2) Very pale. Host's nucleus displaced. Pigment as four or five small masses.

(3) Fairly well-stained protoplasm. No host-nucleus. Pigment as two masses and three granules.

(4) Fairly well-stained protoplasm. Parasite free. Melanin as a number of small masses.

(5) Well-stained protoplasm. Host-nucleus displaced. Melanin as four or five small granules.

(6) Well-stained protoplasm. No host-nucleus. Melanin as a group of granules.

(7) Well-stained protoplasm. Host-nucleus displaced. Melanin as five or six small masses.

(8) Well-stained protoplasm. Host-nucleus displaced. Melanin as a large mass to one side.

(9) Well-stained protoplasm. Host-nucleus displaced. Melanin as two irregular masses.

(10) Well-stained protoplasm. Host-nucleus as a mass and smaller granules.

One of us (T.H.J.) (8) has previously recorded this parasite as *Plasmodium præcox*(?), but a fuller examination has emphasised in our minds the differences then noted between this parasite and the descriptions of the true *P. præcox* available to us. For instance, Minchin(9) points out the bean-shaped character of the gametocytes of *P. præcox*; those of our parasite are practically spherical. That our bodies were for the most part full-grown is evidenced, we think, by red cells being frequently found in which the nucleus had been turned out, and the parasite occupying nearly the whole of the corpuscle. Again, it can hardly be that all the large spherical forms represented schizonts, all exactly at the same stage and ready to form merozoites, but none showing evidence of doing so.

A reference to the description of *Plasmodium majoris* Laveran, in Lühe's(7) article in Mense's "Handbuch der Tropenkrankheiten," shows that the gametocytes of our organisms are only about half the size of the gametocytes of that parasite (5 to 6·5μ as against 11 to 12μ). In the same work, *P. Vaughani* (Novy and McNeal) is stated, from its small size, not to displace the nucleus materially, which our parasite does.

We are, therefore, unable to place our parasite under any of the known forms, which seems to us remarkable in that the common sparrow is not a native of Australia, and we should expect that all its hæmatozoa were known. It may be, of course, that this parasite is not one imported with the sparrow, but acquired from some other source, such as some Australian bird, in which it has not yet been detected. On the other hand, since the sparrow was introduced into Australia many years ago, the descendants of the original pairs must now have reached the 60th or 70th generation; and the descendants of the parasites, if these were imported with the originals, an enormously greater number of generations. It is, therefore, possible, though perhaps improbable, that our parasite represents a variety of *P. præcox*, which has evolved during this period.

Though, as we have elsewhere stated, we are adverse to species-mongering, we think advantage follows the labelling, by specific names, of parasites differing in detail from the description of the type-species: by doing so, attention is called to them, and, when monographing the groups takes place, they can, as future work decides, maintain their rank as true species, or sink their identity under a synonym. We, therefore, propose, tentatively, the name *Plasmodium passeris* for this species.

We have examined a dozen sparrows, finding the hæmatozoon in two. The intestine of one of the birds was infested with a tapeworm, *Monopylidium passerinum*, not previously known from Australia.

The type-slide has been presented to the Australian Museum, and cotypes are being retained by the Bureau.

Spirochaetes in the cæca of *Mus decumanus* and *M. rattus*.

In the rats, *Mus decumanus* Pall., and *Mus rattus* Linn., captured in Sydney, films made from the cæcal contents and stained by Giemsa's method, frequently show numerous very small spirochaetes, uniformly dispersed through the smear or collected more or less in groups. These are necessarily often

almost hidden by the number of bacteria present. The spirochaetes are very small and delicate, with 2 or 3 spirals, sometimes regular, at other times very irregular in their windings, with apparently blunt ends, and usually about 2·5μ long, though occasionally somewhat longer.

Dr. Max Lühe,(7) in Mense's "Handbuch der Tropenkrankheiten," (iii. 1906, p.184), mentions that spirochaetes have been observed in the stomachs of various animals, such as dogs, cats, and "Wanderratte" (*Mus decumanus*). The number of windings is usually 9 to 11, but may be between 2 and 24. This wide variation suggests that the spirochaetes we have met with in the cæcal contents are of a different species whose size is more constant. As they have so far been met with more frequently in *Mus rattus*, we propose the provisional name of *Spirochaeta ratti* for them, for convenience in future reference.

The presence of these spirochaetes in rats is of special interest, in view of the discovery some while ago of spirochaetes in malignant tumours of rodents. Quite recently, these bodies have been found to be in no way etiologically related to the tumours, but accidental associations.

Rounded Bodies, possibly Protozoal, in the Blood-Corpuscles of a Leather-jacket Fish (*Monacanthus* sp.).

(Pl. xlviii., figs.12-13.)

In smear-preparations of the blood of a fish known as a Leather-jacket (*Monacanthus* sp.) obtained at Broughton Island off Port Stephens, N.S.W., by the Director in 1907, and handed to us for examination, many of the corpuscles contained rounded, usually quite circular bodies of various sizes from 0·8μ to 1·5μ in diameter, situated close to but quite separated from the nucleus of the cell. There was a marked contrast in the staining reactions by Giemsa's method between these bodies, the nucleus of the host, and the protoplasm of the host. The latter assumed a greenish-blue tint, the nucleus a deep blue, and the bodies a

pale pink with a less stained centre. Many of the cells contained these bodies; perhaps 1 in 10 did so. In no instance were two found in one red cell.

The corpuscles of this fish (which varied slightly in shape from oval to almost spherical, according, presumably, to the position in which they were fixed) were 8 to 9μ long by 5·5 to 7·5μ broad. In another similar fish caught three days before, and in which the blood-slides were apparently tinted exactly in a similar way, these bodies do not appear.

The nature of these bodies seems uncertain. The fact that, though a considerable percentage of the red cells contained them, in no instance were two found in one cell is rather against their being protozoa. This is important when we consider the comparative frequency of double infections of cells in the Halteridia of birds, and the Plasmodia of birds and man. On the other hand, as these bodies in no way suggest the product of degenerative processes, we are forced to consider them, if not protozoal, as more or less normal products of the cell either when in its younger condition or adult form. This at once suggests that they may represent the centrosomes of the corpuscles which have been recently described by Ronald Ross, Moore, and Walker,(6) in the red corpuscles of the axolotl, the crocodile, man, etc.(Trans. Path. Soc. Lond. Vol.lviii. 1907, p.107). These were described by them as follows in the case of the axolotl :—

"Outside the nuclei, however, one or more small bodies appeared. These were very sharply defined and stained bright red. Very often there were but two, one larger than the other, and frequently they were kidney- or bean-shaped; often, however, there were more than two, sometimes as many as seven or eight, or even more. They were almost invariably close together in one group, frequently connected by filaments, and generally near the nucleus." These bodies, however, are quite unlike the undoubted centrosomes, to be presently described, that we have met with in the red cells of two specimens of Australian tortoises, being larger, more spherical, and less deeply stained.

Sarcosporidia in Pigs, Sheep, Cattle and Rodents.

The following Sarcosporidia have been met with by us. Some of these, we believe, are recorded for the first time in Australia. *Sarcocystis meischeriana* Kühn, in the musculature of pigs in West Australia; *Sarcocystis* (*Balbiania*) *gigantea* Raill., in the œsophagus of sheep in New South Wales and West Australia (in the latter case probably imported from Victoria); *Sarcocystis tenella* Raill., from the muscles of cattle in New South Wales; and *Sarcocystis muris* Blanch., in *Mus rattus* and *M. decumanus* in Sydney.

Trypanosoma lewisi Kent.

As in other parts of the world, *T. lewisi* is not uncommon in *Mus rattus* and *M. decumanus* in Sydney. Its presence has already been recorded in Australia by Pound from Brisbane, and ourselves from Perth, West Australia, and from Sydney.

Centrosomes in the Erythrocytes of Tortoises.

(Plate xlviii., figs.14, 18.)

In the red blood-corpuscles of certain tortoises, *Chelodina longicollis* from Sydney, and *C. oblonga*(?) Gray, from near Perth, West Australia, we have seen bodies resembling the centrosomes described by Ross, Moore and Walker. In the case of the former, the structure consisted of irregularly disposed threads surrounded by a rather lighter staining area (fig.14), whilst in the latter there were four or five well defined masses arranged somewhat like a rosette (fig.18).

Basophile Granulations (Plehn's Bodies) in the Red Corpuscles of Cows suffering from Endemic Hæmaturia of Vesical Origin.

In certain parts near the coast-line of New South Wales, cases of endemic hæmaturia, due to weeping from vascular papillomata of the bladder, are often met with in cattle.(5) No parasitic worms have been found causative of these papillomata, and no

hæmatozoa have been found in the blood. The disease is popularly, and perhaps rightly, considered to be due to the slow action of some vegetable poison.

The loss of blood from these little tumours seems considerable, and a secondary anæmia is the natural consequence. On examining blood-slides from two comparatively early cases in which we were enabled to hold post mortems, the number of red cells with basophile granulations was striking. In the first case, a percentage of about ten were found to contain these, but they were less numerous in the second case. The granules appeared as minute dots, thirty or more in number, uniformly peppering the corpuscle, as fewer but larger very short rods, or as still fewer (about 8 to 10) irregular bodies. The cells containing the many minute dots were usually polychromatophilic, while those with larger bodies were normal in tint. Occasionally unaffected red cells were similarly polychromatophilic.

In addition to these basophile granulations, red cells with nuclei whole or partly disintegrated and absorbed, could be found without difficulty. In all these the nuclei or their remains were stained a deepish purple. The cells with large whole nuclei were usually polychromatophilic. All stages were seen between such nuclei, occupying about two-thirds of the corpuscle, and small often somewhat eccentric deep round purple bodies. Further, other instances were seen in which a large nucleus was irregularly broken up into several irregular masses still in connection with each other, and these were likewise of various sizes.

The basophilic bodies are probably the "chromo-linin granulations," described by C. E. Walker,(6) in the red corpuscles of mammals (Trans. Path. Soc. Lond. lviii. 1907, p.99), evenly distributed throughout the corpuscle. As he points out, these are almost certainly the remains of nuclear matter, and cells containing them can be met with in normal bone-marrow, though they do not appear in the blood under ordinary conditions. When, however, there is a drain upon the blood-system, as in anæmias of various origin, these cells as well as nucleated red cells, with nuclei complete or partially disintegrated, may, in the hurry to

remedy the defect, enter the circulating blood. This would account for the number of the cells with basophile granules in this case, as well as for the increase of nucleated red cells. Further, there is indicated a close relation between the granular cells and the still nucleated ones, the latter being of a still earlier type than the former.

The Director has shown us a photograph of cells with exactly similar basophile granules from Piroplasmosis (Tick Fever) of Cattle, obtained by him several years ago. Here the same conditions causative of their appearance, that is profound anæmia, would be present. Similar cells are occasionally found in man. Dr. Tidswell at the same time reminded us of Türck's view that the granules in question were not nuclear, but arose from the cytoplasm.

LITERATURE.

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2. Balfour—Journ. Trop. Med. Hyg. ix., March, 1906; Second Report Wellcome Research Lab. Khartoum, 1906, p.110.
3. Johnston—Agric. Gazette, N.S. Wales, xx., 1909, p.581; Proc. Linn. Soc. N. S. Wales, 1909, xxxiv., p.218.
4. Cleland and Johnston—Journ. Proc. Roy. Soc. N. S. Wales, xliii., 1909, p.75.
5. Cleland—Proc. Linn. Soc. N. S. Wales, 1909, xxxiv., p.216.
6. Ross, Moore, and Walker—Trans. Path. Soc. Lond. lviii., 1907, p.99.
7. Luehe, in Mense's "Handbuch der Tropenkrankheiten," iii., 1906, p.184, etc.
8. Johnston—Rec. Austr. Mus. vii., 1909, p.344.
9. Minchin, in Ray Lancaster's Treatise on Zoology, Pt.1, fasc. ii., 1903, p.268.

EXPLANATION OF PLATE XLVIII.

Figs.1-11.—Mononuclear leucocytes of *Mus decumanus* containing *Leucocytozoon muris* Balfour.

Figs.1,4-10.—Ditto, from heart-blood.

Figs.2,3,11.—Ditto from liver-smear.

Figs.1,5,6,7,9.—Encapsuled forms.

Figs.2,4.—Host-nucleus partly divided by parasite.

Fig. 3.—Host-nucleus quite divided by parasite.
Fig. 10.—Encapsuled form free in plasma (blood from heart).
Fig. 11.—Encapsuled form free in plasma (blood from liver).
Figs. 12,13.—Erythrocytes of *Monacanthus* sp., showing "ring" bodies.
Fig. 14.—Erythrocytes of *Chelodina longicollis* with centrosome.
Figs. 15-17.—*Halteridium nettionis* in erythrocytes of *Nettion castaneum*.
Fig. 15.—Male form.
Fig. 16.—Female form.
Fig. 17.—Young intermediate form.
Fig. 18.—Erythrocyte of *Chelodina oblonga* showing centrosome.
Figs. 19-24.—*Plasmodium passeris* in erythrocytes of *Passer domesticus*.
Fig. 19.—Young amoeboid non-pigmented parasite.
Fig. 20.—Double infection by young forms.
Figs. 21,22.—Adult form, host-nucleus displaced.
Fig. 23.—Adult form, host-nucleus expelled.
Fig. 24.—Young pigmented form.
Fig. 25.—*Spirochaeta ratti* from cæcum of *Mus rattus*.

References to lettering:—*n.*, host-nucleus—*p.*, parasite—*c.*, capsule of parasite.

All figures have been drawn, using the same magnification.